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MINIREVIEW

When Two Strands Are Better Than One: The Mediators and Modulators of the Cellular Responses to Double-Stranded RNA

BERTRAM L. JACOBS[1] and JEFFREY O. LANGLAND

Department of Microbiology and the Graduate Program in Molecular and Cellular Biology, Arizona State University, Tempe, Arizona 85287-2701

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Double-stranded RNA is a potent inducer of interferon, a modulator of the expression of a number of other genes involved in the response of cells to virus infection, an activator of the interferon-induced antiviral state, and may be involved in differentiation, induction of apoptosis, and control of oncogenic transformation. This review will attempt to summarize what is known about the cellular proteins that act to mediate the response of cells to double-stranded RNA and the viral and cellular macromolecules that may be able to modulate these responses.

THE PLAYERS

Most viruses induce the synthesis of dsRNA[2] at some time during their replication cycle. The presence of viral dsRNA appears to trigger many of the cellular responses to virus-infection, probably through activation of dsRNA-dependent enzymes, including the interferon-inducible protein kinase, PKR, and the interferon-inducible enzyme, 2′,5′ oligoadenylate synthetase. As countermeasures, many viruses have evolved mechanisms to mask the effects of dsRNA on cells. This review will first summarize what is known about synthesis of dsRNA in virus-infected (and perhaps uninfected) cells, will then tackle the dsRNA-activatable enzymes that are present in cells, and finally will review the viral and cellular products that can modulate the cellular response to dsRNA.

SOURCES OF dsRNA

Before discussing the macromolecules in cells that mediate the response to dsRNA it is important to get a glimpse of the sources of RNA that can interact with these molecules in infected and perhaps uninfected cells. Actually identifying the potential sources of dsRNA in infected cells has in fact been problematic over the years. Minute quantities of dsRNA, as little as one molecule per cell (Marcus and Sekellick, 1977), can have profound effects on cellular physiology. It is difficult to detect such miniscule quantities of preexisting dsRNA in cell extracts. This problem is exacerbated by the potential reassociation of complementary strands of RNA during extract preparation, especially during extraction with phenol (Kohne *et al.*, 1977), always calling into question whether any isolated dsRNA preexisted in the cell or was in fact an artifact of reassociation during isolation. Nonetheless, binding of anti-dsRNA antibodies to viroplasm of extracted whole cells (Lee *et al.*, 1994a) and isolation of viral mutants that alter activation of dsRNA-dependent processes (Kitajewski *et al.*, 1986; Beattie *et al.*, 1991, 1995a,b; Chang *et al.*, 1995) all suggest that dsRNA does in fact exist within virus-infected cells.

For viruses with dsRNA genomes the obvious suspect is the genome itself. However, completely uncoated dsRNA genome has not been detected in cells infected with dsRNA-viruses (Schiff and Fields, 1990). For the reoviruses, input viral dsRNA remains within the inner capsid throughout the viral life cycle, and progeny genome is only synthesized after assembly of positive sense single-stranded progeny RNA into subviral particles. It is likely that the machinations that dsRNA viruses go through to prevent exposure of naked dsRNA in cells is a consequence of the profound effects that dsRNA has on the physiology of the cell. Nonetheless, it could be that minute amounts of incorrectly uncoated or packaged genome might be present in infected cells and could activate the known dsRNA-dependent enzymes. Alterna-

[1] To whom correspondence and reprint requests should be addressed. Fax: (602)965-0098. E-mail (Internet): idblj@asuvm.inre.asu.edu.

[2] Abbreviations used: DRAD, dsRNA adenosine deaminase; dsRNA, double-stranded RNA; E3L, the vaccinia virus gene coding for a dsRNA-binding protein inhibitor of PKR; EBER, Epstein–Barr virus-encoded small RNA; eIF-2, eukaryotic protein synthesis initiation factor 2; K3L, the vaccinia virus gene coding for an eIF-2α homologue inhibitor of PKR; PKR, the interferon-inducible, dsRNA-dependent protein kinase; RRE, the HIV rev responsive element; tar, the HIV tat responsive element; tat, the HIV transactivator of gene expression; VAI RNA, adenovirus-encoded small RNA I; VAII RNA, adenovirus-encoded small RNA II.

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tively, secondary structure on mRNA might be involved in activating these enzymes in infected cells. Such structure has been implicated in the poor translation of the reovirus s1 mRNA (Henry *et al.*, 1994).

For ssRNA viruses the obvious culprit is replicative intermediate in infected cells. This is supported by the isolation of both positive and negative sense RNA bound to the dsRNA-binding protein 2′,5′ oligoadenylate synthetase from EMCV-infected cells (Gribaudo *et al.*, 1991) and by binding of antisera that recognizes dsRNA to viroplasm in rubella and semliki forest virus-infected cells (Lee *et al.*, 1994a). Viral dsRNA isolated from influenza virus-infected lungs was able to induce both the local and systemic cytotoxic effects typical of influenza virus-infection when injected into experimental animals (Majde *et al.*, 1991; Kimura *et al.*, 1992). Again, however, it is unclear how much true dsRNA, i.e., full duplexes between positive and negative sense RNA, exists in cells infected with ssRNA viruses.

For DNA viruses dsRNA appears to accumulate as the result of overlapping convergent transcription. At late times after infection with vaccinia virus, viral transcripts fail to terminate at discrete sites at the ends of genes (Moss, 1990). Thus, complementary mRNAs are produced from genes transcribed in opposing directions (Colby and Duesberg, 1969; Duesberg and Colby, 1969; Colby *et al.*, 1971; Boone *et al.*, 1979). The vaccinia virus A18R gene product modulates transcription termination, thereby altering the level of dsRNA that accumulates in infected cells, with defects in A18R leading to excess dsRNA (Bayliss and Condit, 1993; Simpson and Condit, 1994). Similar complementary transcripts have been detected in adenovirus- (Pettersson and Philipson, 1974; Maran and Mathews, 1988), herpes simplex virus- (Jacquemont and Roizman, 1975), and SV-40- (Aloni, 1972) infected cells. Again, the actual extent of hybridization between these complementary strands in infected cells is unclear, although a large fraction of the RNA in vaccinia virus-infected cells is ribonuclease resistant even before deproteination (Colby *et al.*, 1971). In addition, activation of the dsRNA-dependent pathways has been detected in cells infected with either adenovirus (Kitajewski *et al.*, 1986) or vaccinia virus (Beattie *et al.*, 1991, 1995a,b; Chang *et al.*, 1995) deleted for inhibitors of these pathways.

While fully duplexed RNA has not been detected in retrovirus-infected cells, the HIV and HTLV genomes contain large domains of secondary structure (*tar* and *RRE/RxRE*) that function to regulate gene expression and can interact with several dsRNA-binding proteins (Sengupta and Silverman, 1989; Edery *et al.*, 1989; Schroder *et al.*, 1990; Silverman and Sengupta, 1990; Gunnery *et al.*, 1990, 1992; Gatignol *et al.*, 1993; Park *et al.*, 1994; Maitra *et al.*, 1994).

Perhaps some of the most intriguing findings have been the detection of dsRNA-like molecules in apparently uninfected cells (Maran and Mathews, 1988; Li and Petryshyn, 1991), which are capable of activating the dsRNA-dependent protein kinase, PKR, *in vitro*. This RNA is apparently poly(A) rich and may contain topologically unlinked complementary strands. Again, it is unclear if these hybrids exist in cells or hybridize during RNA isolation, although PKR activation has been detected in differentiating adipocytes (Li and Petryshyn, 1991), from which dsRNA can be isolated, and after IL-3 deprivation of an IL-3-dependent murine cell line (Ito *et al.*, 1994).

MEDIATORS OF THE CELLULAR RESPONSE TO dsRNA

Two of the cellular gene products whose activities are most clearly regulated by dsRNA are the protein kinase, PKR, and the enzyme 2′,5′ oligoadenylate synthetase (Sen and Lengyel, 1992; Pestka *et al.*, 1987). Both enzymes can be induced in cells treated with interferon, and both enzymes can bind to and be potently activated at a posttranslational step by dsRNA. In the case of PKR, activation occurs concomitantly with intermolecular (Kostura and Mathews 1989; Thomis and Samuel 1995) and perhaps intramolecular (Berry *et al.*, 1985; Galabru *et al.*, 1989) phosphorylation, which may be accompanied by dimerization (Langland and Jacobs, 1992; Patel *et al.*, 1995). The level of PKR activation may be regulated in cells by depletion of Ca^{+2} stores in the endoplasmic reticulum (Prostko *et al.*, 1995). Once activated, PKR can phosphorylate a number of exogenous substrates including the small (α) subunit of the protein synthesis initiation factor eIF-2 (Farrell *et al.*, 1977; Levin and London, 1978; Samuel, 1979), the NFκB inhibitor IκB (Maran *et al.*, 1994; Kumar *et al.*, 1994; Offermann *et al.*, 1995), and histone proteins (Jacobs and Imani, 1988; Galabru and Hovanessian, 1985). eIF-2α phosphorylation can lead to an inhibition of the initiation of protein synthesis. eIF-2α phosphorylation by PKR is presumed to be involved in the interferon-mediated inhibition of replication of a number of viruses. Constitutive expression of either human (Meurs *et al.*, 1992) or mouse (Baier *et al.* 1993) PKR leads to an inhibition of replication of EMCV, but not VSV. For both adenovirus (Kitajewski *et al.* 1986) and vaccinia virus (Beattie *et al.*, 1991, 1995a,b; Chang *et al.*, 1995), deletion of inhibitors of PKR (VAI RNA for adenovirus and the E3L or K3L genes for vaccinia virus) leads to increased phosphorylation of eIF-2α and renders these normally interferon-resistant viruses sensitive to the effects of interferon. In the case of adenovirus, deletion of the VAI gene can be complemented by overexpression of a nonphosphorylatable variant of eIF-2α (Davies *et al.*, 1989). Phosphorylation of IκB by PKR can lead to IκB degradation and subsequent activation of NFκB (Maran *et al.*, 1994; Kumar *et al.*, 1994; Offermann *et al.*, 1995), perhaps through a reactive oxygen-mediated pathway (Schreck *et al.*, 1991). The activation of NFκB mediated

by dsRNA may be involved in induction of interferon-β gene expression and of the other cellular genes whose transcription is influenced by dsRNA. PKR-mediated phosphorylation of histone proteins has only been detected *in vitro* and its biological significance is at present unclear. Activated PKR may also be able to induce apoptosis in vaccinia virus-infected cells (Lee and Esteban, 1994), although neither the activators nor substrates involved in this response have been characterized. Inhibition of endogenous PKR, either by expression of dominant negative mutants of PKR (Koromilas *et al.*, 1992; Meurs *et al.*, 1993) or by expression of a natural cellular inhibitor of PKR (Barber *et al.*, 1994) produced a transformed phenotype in cells, as did overexpression of a nonphosphorylatable mutant of eIF-2α (Donze *et al.*, 1995). PKR has been found in most mammalian cells analyzed. An analogous enzyme, which is immunologically cross-reactive with human PKR, has been identified in plant cells (Langland *et al.*, 1995) and is inducible by virus and viroid infection (Crum *et al.*, 1988; Hiddinga *et al.*, 1988; Roth and He, 1994).

Activation of 2′,5′ oligoadenylate synthetase by dsRNA is likely not a consequence of a posttranslational modification, but of a conformational change in the enzyme induced by binding to dsRNA. Once activated, the enzyme can polymerize ATP and other nucleotides in novel 2′,5′ linkages (Kerr and Brown, 1978). These 2′,5′ oligoadenylates can activate a ribonuclease, RNase L, that can cleave ssRNAs, including rRNA, at UpA, UpG, or UpU residues (Silverman *et al.*, 1988; Bisbal *et al.*, 1989; Baglioni *et al.*, 1979; Nilsen *et al.*, 1982; Schroder *et al.*, 1989; Floyd-Smith *et al.*, 1981; Wreschner *et al.*, 1981). Several isoforms of 2′,5′ oligoadenylate synthetase have been identified, and cDNA clones for two of the isoforms have been characterized (Laurent *et al.*, 1983; Yang *et al.*, 1981; Chebath *et al.*, 1987a; Saunders *et al.*, 1985; Ilson *et al.*, 1986; Rosenblum *et al.*, 1988; Marie *et al.*, 1990; Wathelet *et al.*, 1986). The smaller of the two isoforms of 2′,5′ oligoadenylate synthetase appears to be sufficient to inhibit replication of EMCV and vaccinia virus but not VSV in cells transfected with plasmid engineered to constitutively express the enzyme (Chebath *et al.*, 1987b; Grun *et al.*, 1987). In addition, expression of antisense RNA to the small isoform of 2′,5′ oligoadenylate synthetase prevented the interferon-mediated inhibition of EMCV replication (Benedetti *et al.*, 1987), as did expression of a dominant negative mutant of RNase L (Hassel *et al.*, 1993). Both negative and positive sense picornaviral RNA have been found bound to 2′,5′ oligoadenylate synthetase in EMCV-infected cells (Gribaudo *et al.*, 1991), consistent with activation of the pathway in picornavirus-infected cells.

Both PKR and 2′,5′ oligoadenylate synthetase bind specifically to dsRNA or RNA with secondary structure, including the reovirus s1 mRNA (Bischoff and Samuel, 1989), adenovirus VAI RNA (Desai *et al.*, 1995; Mathews and Shenk, 1991), and the *tar* region of the HIV-1 RNA (Maitra *et al.*, 1994; Silverman and Sengupta, 1990; Gunnery *et al.*, 1990, 1992; Edery *et al.*, 1989; Sengupta and Silverman, 1989; Schroder *et al.*, 1990). PKR binds to RNA with K_ds in the nM range (McCormack and Samuel, 1995; Schmedt *et al.*, 1995). For PKR, the amino-terminal third of the protein appears to be both necessary and sufficient for binding to dsRNA (McCormack *et al.*, 1992; Patel and Sen, 1992; Feng *et al.*, 1992; Katze *et al.*, 1991). This region contains two copies of a motif that has been found in a number of proteins that bind specifically to dsRNA or structured RNA (St. Johnston *et al.*, 1993; Chang *et al.*, 1992; Chang and Jacobs, 1993; McCormack *et al.*, 1992). Both copies of the motif appear necessary for high affinity binding of PKR to dsRNA, although the amino-proximal motif seems to be more important for binding and activity than the internal motif. Mutation of several residues in PKR, conserved among most proteins containing the motif, interfered with binding to dsRNA (McCormack *et al.*, 1994; Green *et al.*, 1995; Romano *et al.*, 1995; McMillan *et al.*, 1995; Clarke and Mathews, 1995).

The dsRNA-binding domain for the 2′,5′ oligoadenylate synthetases has not been as well defined as the domains on PKR. Sequences within the amino-terminal 158 residues on the small (42-kDa) isoform of 2′,5′ oligoadenylate synthetase are necessary for binding to dsRNA (Ghosh *et al.*, 1991). This region of 2′,5′ oligoadenylate synthetase contains no sequences homologous to any other proteins in the database. The cDNA clone of the larger, 69-kDa isoform of 2′,5′ oligoadenylate synthetase contains a duplication of sequences homologous to the small isoform of 2′,5′ oligoadenylate synthetase (Marie and Hovanessian, 1992). As of yet the sequences necessary for the 69-kDa isoform to bind to dsRNA have not been identified.

Both PKR and the various forms of 2′,5′ oligoadenylate synthetase have been found in the cytoplasm as well as the nucleus (Jimenez Garcia *et al.*, 1993; Jeffrey *et al.*, 1995; Saunders *et al.*, 1985; Rosenblum *et al.*, 1988) of cells. For PKR approximately 80% of the enzyme is found in the cytoplasm while 20% is found in the nucleus (Jeffrey *et al.*, 1995). The nuclear form of the enzyme was concentrated in nucleoli and was apparently relatively underphosphorylated, compared to cytoplasmic enzyme. The cytoplasmic form of PKR is both associated with ribosomes (80%) and free in the cytoplasm (20%). Cytoplasmic PKR not bound to ribosomes is partially phosphorylated and a dimer (Langland and Jacobs, 1992). The different roles that these differentially localized and differentially phosphorylated forms of the kinase play in inhibition of virus replication or in normal cell physiology is at present unclear.

VIRAL MODULATORS OF THE RESPONSE TO dsRNA

Given the efficiency of the dsRNA-dependent enzymes at inhibiting virus infection, it is perhaps not surprising

that a number of viruses have evolved pathways to counteract activation and/or activity of both 2′,5′ oligoadenylate synthetase and PKR. The most well-characterized PKR inhibitor is the adenovirus VAI RNA (Mathews and Shenk, 1991). VAI RNA is found in abundant amounts at late times after infection with adenovirus. A second adenovirus-encoded small RNA, VAII RNA, is synthesized in lower amounts in infected cells. Virus deleted for VAI replicates poorly, while virus deleted for VAII replicates as well as wild-type virus (Thimmappaya *et al.*, 1982). VAI RNA is highly structured in solution (Furtado *et al.*, 1989; Mellits and Mathews, 1988) and binds to PKR in competition with dsRNA (Galabru *et al.*, 1989; Katze *et al.*, 1987; Kostura and Mathews, 1989; Mellits *et al.*, 1990) but fails to efficiently lead to PKR autophosphorylation or activation. The ability to bind to PKR in competition with dsRNA is not sufficient for VAI function, since several mutants of VAI that bind PKR *in vitro* do not effectively support adenovirus replication (Mellits *et al.*, 1990). Binding to PKR requires an apical stem structure of at least half a turn in length (Clarke *et al.*, 1994), but a central domain of complex structure is required to inhibit PKR activation (Pe'ery *et al.*, 1993; Ghadge *et al.*, 1994). Deletion of the VAI gene leads to a virus that replicates poorly and is sensitive to the antiviral effects of interferon (Thimmappaya *et al.*, 1982; Kitajewski *et al.*, 1986). Analogous Epstein–Barr virus-encoded structured small RNAs (EBERs) can inhibit activation of PKR (Clarke *et al.*, 1991; Sharp *et al.*, 1993; Clemens *et al.*, 1994). The Epstein–Barr virus encoded small RNAs can at least partially complement adenovirus deleted for VAI RNA (Bhat and Thimmappaya, 1983) as can SV-40 large T antigen (Rajan *et al.*, 1995). However, the role of at least the Epstein–Barr virus encoded small RNAs in infected cells is unclear since deletion of the gene for these RNAs has no effect on sensitivity of the virus to interferon treatment (Swaminathan *et al.*, 1992). EBERs have been shown to bind to the ribosomal protein L22 (Toczyski *et al.*, 1994). Again, the functional significance of binding to L22 is at present unclear. VAI RNA can interact with 2′,5′ oligoadenylate synthetase, but activates rather than inhibits the enzyme (Desai *et al.*, 1995). As is true of many of the macromolecules described in this review, VAI RNA could be detected in the nucleus as well as the cytoplasm of infected and transfected cells (Jimenez Garcia *et al.*, 1993). The role that nuclear localization of VAI RNA plays in virus replication is at present unclear.

Vaccinia virus-infected cells contain at least two products capable of inhibiting PKR. The protein product of the K3L gene has partial homology to one of the substrates of PKR, eIF-2α (Beattie *et al.*, 1991; Goebel *et al.*, 1990), and can inhibit phosphorylation of this protein synthesis initiation factor (Carroll *et al.*, 1993; Davies *et al.*, 1992, 1993; Jagus and Gray, 1994). The K3L gene product is thought to function by binding to PKR competitively with eIF-2 (Carroll *et al.*, 1993; Jagus and Gray, 1994). Virus deleted for K3L is interferon sensitive (Beattie *et al.*, 1991), but does not have a host range different from wild-type vaccinia virus (see below) and does not induce apoptosis in infected cells (Lee and Esteban, 1994).

The vaccinia virus E3L gene codes for a second modulator of dsRNA in virus-infected cells. The E3L gene codes for two proteins that can both bind specifically to dsRNA (Watson and Jacobs, 1991; Chang *et al.*, 1992; Yuwen *et al.*, 1993). Cloned E3L gene products can inhibit activation of PKR (Chang *et al.*, 1992). Inhibition *in vitro* appears not to be catalytic (Whitaker-Dowling and Youngner, 1983; Jagus and Gray, 1994) and can be overcome with increasing concentrations of dsRNA (Whitaker-Dowling and Youngner, 1983; Watson and Jacobs, 1991). Deletion of the E3L gene from vaccinia virus leads to loss of kinase inhibitory activity and to degradation of rRNA characteristic of activation of the 2′,5′ oligoadenylate synthetase/RNase L pathway (Beattie *et al.*, 1995a). Virus deleted for E3L has a host-range phenotype, in that it will replicate in RK-13 and CEF cells but not in HeLa or Vero cells (Beattie *et al.*, 1995a,b; Chang *et al.*, 1995). Replication in L cells is semipermissive (Beattie *et al.*, 1995). In those cells in which virus deleted for E3L does replicate, replication is sensitive to the anti-viral effects of interferon (wild-type vaccinia virus is resistant to the effects of interferon in most cell types tested) (Beattie *et al.*, 1995a; Chang *et al.*, 1995). Virus deleted for E3L induces apoptosis in HeLa cells (Lee and Esteban, 1994). Thus, the host-range phenotype may be a consequence of induction of apoptosis in cells. Induction of apoptosis by virus deleted for E3L, together with the fact that PKR expressed from wild-type vaccinia virus induces apoptosis (Lee and Esteban, 1994), suggests that viral dsRNA may be inducing apoptosis by activating PKR. Results on influenza virus-mediated induction of apoptosis suggest that dsRNA might mediate its effects by induction of fas antigen, again, perhaps through activation of PKR (Takizawa *et al.*, 1995). Several other proteins that can bind to dsRNA can substitute for E3L in allowing replication in HeLa cells (Park *et al.*, 1994; Langland *et al.*, 1994; Beattie *et al.*, 1995a). Interestingly, the E3L gene products are detected primarily in the nucleus at early times after infection and in transfected cells (Yuwen *et al.*, 1993). The role that migration of these gene products to the nucleus plays in virus replication is at present unclear, although a mutant of E3L encoding a protein that fails to migrate to the nucleus could rescue the host-range defect of vaccinia virus deleted for E3L (Chang *et al.*, 1995).

The vaccinia virus E3L gene contains a single copy of the dsRNA-binding motif also found in PKR (Chang *et al.*, 1992). Deletion and point mutation analysis indicates that this motif is necessary for binding of the E3L gene products to dsRNA (Chang and Jacobs, 1993). The ability of mutants of E3L to support replication of vaccinia virus in HeLa cells correlates with their ability to bind dsRNA

(Chang *et al.*, 1995). A similar motif is found in the porcine group C rotavirus NSP3 gene (Langland *et al.*, 1994). While a direct mutational analysis of NSP3 gene function is not possible due to the lack of a gene replacement system in rotaviruses, the NSP3 gene can fully complement deletion of the E3L gene in vaccinia virus. The NSP3 gene is predicted to encode a 45-kDa protein. However, when NSP3 is expressed either *in vitro*, or in transfected cells, the 45-kDa product is cleaved into 38- and 8-kDa proteins. The 8-kDa protein contains the dsRNA-binding motif and binds to dsRNA. This 8-kDa polypeptide has been detected in cells infected with group C rotaviruses (Langland *et al.*, 1994), and thus is the smallest known natural protein that can specifically bind dsRNA and antagonize at least some of the effects of dsRNA.

The reovirus $\sigma 3$ protein can also bind specifically to dsRNA (Huismans and Joklik, 1976), even though it has, at best, limited homology to other known dsRNA-binding proteins. $\sigma 3$ can inhibit PKR activation *in vitro* (Imani and Jacobs, 1988) and in transfected cells (Giantini and Shatkin, 1989; Lloyd and Shatkin, 1992; Denzler and Jacobs, 1994). The gene encoding $\sigma 3$ (S4) can also partially complement deletion of E3L from vaccinia virus (Beattie *et al.*, 1995a) and deletion of VAI from adenovirus (Lloyd and Shatkin, 1992). Some strains of reovirus are relatively resistant to the effects of interferon while other strains are quite sensitive (Jacobs and Ferguson, 1991). As of yet the gene encoding resistance of reovirus to interferon has not been identified. Strains of reovirus also differ in their ability to inhibit translation of host mRNAs. The ability of reovirus to inhibit host protein synthesis maps to the gene encoding $\sigma 3$ (Sharpe and Fields, 1982). While all strains of mammalian reovirus thus far analyzed code for a $\sigma 3$ protein capable of binding dsRNA (Seliger *et al.*, 1992), there may be strain differences in the affinity of $\sigma 3$ for dsRNA, in the amount of $\sigma 3$ made in infected cells, or in the ability of $\sigma 3$ to bind to another viral protein, $\mu 1$, which can abrogate binding of $\sigma 3$ to dsRNA. Binding to dsRNA has been mapped to a basic region in the carboxy-half of the protein (Schiff *et al.*, 1988; Miller and Samuel, 1992; Denzler and Jacobs, 1994; Mabrouk *et al.*, 1995). Mutations of the protein in this domain that inhibit dsRNA-binding fail to support replication of vaccinia virus deleted for E3L in HeLa cells (Beattie *et al.*, 1995a).

In addition to the specific dsRNA-binding noted for the E3L gene products and $\sigma 3$, several viral proteins appear to bind to several different nucleic acids, including dsRNA. The influenza virus NS-1 protein can bind to both negative sense influenza virus RNA (Hatada *et al.*, 1992) and dsRNA (Hatada and Fukuda, 1992). NS-1 protein can act as an inhibitor *in vitro* of PKR and can block the dsRNA-mediated inhibition of translation *in vitro* (Qian *et al.*, 1995; Lu *et al.*, 1995). In addition, mutants of NS-1 have been shown to alter translation of viral RNAs in infected cells, and NS-1 expressed in HeLa cells can stimulate translation of reporter mRNAs containing 5′ untranslated regions of influenza virus mRNAs (Enami *et al.*, 1994). The hantavirus core protein appears also to bind to ssRNA and dsRNA (Gott *et al.*, 1993), while the reovirus $\lambda 1$ protein can bind to either dsRNA or dsDNA (Lemay and Danis, 1994).

HIV-infected cells contain a number of macromolecules capable of modulating the activity of PKR. As indicated previously, the *tar* stem–loop structure at the 5′-end of HIV genome RNA and mRNA can bind to PKR (Maitra *et al.*, 1994; Silverman and Sengupta, 1990; Gunnery *et al.*, 1990, 1992; Edery *et al.*, 1989). PKR binds to *tar* with 100-fold lower affinity than to either fully duplexed RNA or VAI RNA (McCormack and Samuel, 1995). Alternative investigators have argued that *tar* RNA sequences can either activate (Maitra *et al.*, 1994; Edery *et al.*, 1989) or antagonize (Gunnery *et al.*, 1992) activation of PKR. Interaction of PKR with *tar* could be inhibited by tat protein (Judware *et al.*, 1993).

CELLULAR MODULATORS OF THE RESPONSE TO dsRNA

Uninfected cells also contain a number of factors capable of modifying the effects of dsRNA. Human, bovine, mouse, and monkey cells contain a latent inhibitor of PKR, termed P58 (Lee *et al.*, 1990, 1992, 1994b; Lee and Katze, 1994; Barber *et al.*, 1994). The latent inhibitor could be separated from an "anti-inhibitor" either by precipitation with ammonium sulfate or by infection with influenza virus (Lee *et al.*, 1990). Active inhibitor decreases both PKR activation and activity toward eIF-2α, in an unknown manner (Lee *et al.*, 1990). The gene for P58 has been cloned, sequenced, and expressed. The protein is a member of a family of proteins, called the tetratricopeptide family, which includes cdc23, cdc16, and bimA, that may be involved in regulation of the cell cycle (Barber *et al.*, 1994; Lee *et al.*, 1994b). Overexpression of P58 results in transformation of cells in culture, presumably by inhibiting endogenous PKR (Barber *et al.*, 1994; Lee *et al.*, 1994b).

Uninfected cells also contain an eIF-2 associated protein, called p67 (Datta *et al.*, 1989) that can block PKR-mediated phosphorylation of either eIF-2α or histone proteins (Ray *et al.*, 1992). p67 has also been reported to block autophosphorylation of PKR (Ray *et al.*, 1992). p67 may be a general inhibitor of eIF-2α phosphorylation in that it can prevent phosphorylation mediated by the heme regulated eIF-2α kinase (Ray *et al.*, 1993). The inhibitor is degraded in serum-starved cells and its synthesis is induced by subsequent mitogen treatment (Ray *et al.*, 1992). Activity of the inhibitor may also be regulated by deglycosylation (Datta *et al.*, 1989).

Undifferentiated preadipocytes contain a 15-kDa protein inhibitor of PKR (Judware and Petryshyn, 1991, 1992). The inhibitor appears to block interaction of PKR

with dsRNA, but does not interact with dsRNA activator (Judware and Petryshyn, 1992). It has been suggested that the inhibitor binds directly to PKR to block its interaction with dsRNA.

The human cellular protein, TRBP (Gatignol *et al.*, 1991), has been shown to bind to dsRNA (Gatignol *et al.*, 1993; Park *et al.*, 1994) and to inhibit activation of PKR *in vitro* (Park *et al.*, 1994). TRBP contains three copies of the dsRNA-binding motif (Gatignol *et al.*, 1993; Park *et al.*, 1994). Overexpression of TRBP can complement the host-range defect of vaccinia virus deleted for the E3L gene (Park *et al.*, 1994). The role of TRBP in uninfected and in virus-infected cells is at present unclear, although human TRBP can bind to *tar* and RRE sequences on HIV RNA (Gatignol *et al.*, 1991, 1993; Park *et al.*, 1994) and can coimmunoprecipitate HIV RNA from infected cells (Gatignol *et al.*, 1993). A similar protein has been identified in *Xenopus levis* cells (St. Johnston *et al.*, 1993).

An adenosine deaminase that uses dsRNA as a substrate (dsRNA adenosine deaminase; DRADA) has been identified in a number of cells (O'Connell and Keller, 1994; Kim *et al.*, 1994; Hough and Bass, 1994; Morrissey and Kirkegaard, 1991; Nishikura, 1992). The inosine formed by deamination of adenosine base pairs with cytosine rather than uridine, with two consequences. First, adenosine deamination destabilizes the RNA:RNA duplexes and, second, transcription of deaminated strands leads to insertion of cytosine residues rather than uridine residues and thus to hypermutation. This enzyme likely plays a role in RNA editing (Kim and Nishikura, 1993) and is likely responsible for hypermutation of certain viruses (Cattaneo, 1994), including measles virus isolated from patients with SSPE (Cattaneo and Billeter, 1992). Secondary structure in the *tar* region of the HIV-1 RNA is also a substrate for *Xenopus* DRADA (Sharmeen *et al.*, 1991), although it is unclear if HIV RNA interacts with this enzyme in infected cells.

Double-stranded RNA also seems likely to be involved in signal transduction mediated by the ras oncogene. v-Ras transformation of cells induces an inhibitor of PKR (Mundschau and Faller, 1992). The inhibitor is heat and organic solvent sensitive, suggesting that it contains a protein as a necessary component (Mundschau and Faller, 1992). The inhibitor migrates through gel filtration chromatography with a M_r of approximately 100,000 (Mundschau and Faller, 1994). The inhibitor acted *in trans* to prevent phosphorylation of PKR, through an as yet unknown mechanism. The v-ras-induced inhibitor of PKR may interfere with PDGF induction of immediate early response genes, suggesting that PDGF may function through activation of PKR (Mundschau and Faller, 1995).

Finally, *La* antigen can bind dsRNA (Xiao *et al.*, 1994) and EBER RNAs (Lerner *et al.*, 1981), as well as snRNAs, can unwind dsRNA, and can inhibit activation of PKR *in vitro* (Xiao *et al.*, 1994). Histone proteins, in addition to being substrates for PKR, bind to dsRNA as well as to dsDNA and can prevent activation of PKR *in vitro* (Jacobs and Imani, 1988). Given the recent identification of PKR in the nucleus of cells these proteins may be acting as inhibitors and/or substrates of PKR in cells.

CONCLUDING REMARKS

The role of dsRNA in the response of cells to virus infection has been evident for a number of years. Our increased understanding of the resources that viruses invest to protect themselves from this response adds credence to the critical role of dsRNA in the cells recognition and response to virus infection. The potential role of dsRNA in uninfected cells is just beginning to be gleaned. The future will no doubt see great progress in our understanding of the interaction between cellular RNAs and the dsRNA-activated machinery in cells, progress that will almost certainly utilize the reagents that viruses have given to us as probes.

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